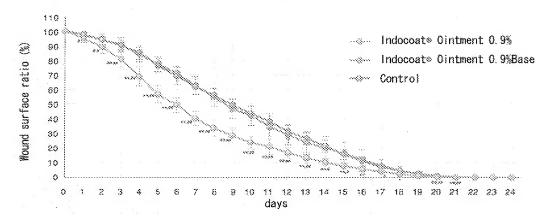
# Promoting Material of MARUHO

page 5: Treatment effect in a rat bedsore model <sup>1)</sup> [Wound surface ratio\*]

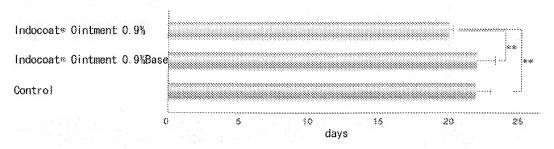
In a group of Iodocoat<sup>®</sup> Ointment 0.9%, a significant reduction of wound surface ratio was observed on 2 to 16, 18, 20 and 21 days compared to a control group. A significant reduction of wound surface ratio was also observed on 1 to 15 and 17, 20 and 21days compared to a group of Iodocoat<sup>®</sup> base.



\*wound surface ratio(%) =

# [Days for treatment]:

In a group of Iodocoat<sup>®</sup> Ointment 0.9%, a significant reduction of days for treatment was observed compared to a control group or a group of Iodocoat<sup>®</sup> base.



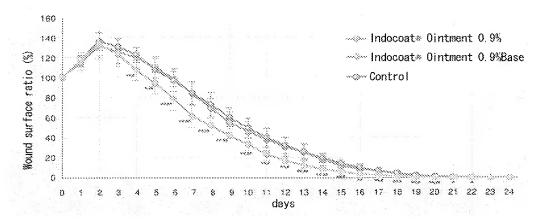
Mean  $\pm$  S.D. \*\*p<0.01 (Tukey's test)

Method: A test medicine was applied with an amount of 150 mg/once a day to a rat experimental bedsore model, which is corresponding to a bedsore of degree III. Transient ratio of wound surface and days for treatment were observed until epithelization was completed. As a control, a gauze soaked in sterile physiological saline was applied.

- 1) a rat bedsore model: The back of a rat was dehaired, and loaded under pressure for 24 hours under anesthesia. Dead skin was surgically removed 2 days after pressure was removed.
- 2) a bedsore of degree III: a model in which denaturation was reached to the fatty tissue, but not to the muscle membrane.

page 6:
Healing effect in a rat bedsore model <sup>1)</sup>
[Wound surface ratio\*]

In a group of Iodocoat<sup>®</sup> Ointment 0.9%, a significant reduction of wound surface ratio was observed on 4 to 22 days compared to a control group. A significant reduction of wound surface ratio was also observed on 4 to 15 and 17 to 21days compared to a group of Iodocoat<sup>®</sup> base.

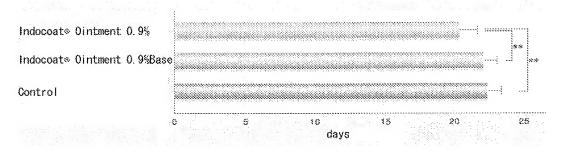


\*wound surface ratio(%) =

#### [Days for treatment]:

In a group of Iodocoat® Ointment 0.9%, a significant reduction of days for

treatment was observed compared to a control group or a group of Iodocoat® base.



Mean  $\pm$  S.D. \*\*p<0.01 (Tukey's test) n=10

Method: A test medicine was applied with an amount of 150 mg/once a day to a rat experimental burn model, which is corresponding to a burn wound of degree III <sup>2)</sup>. Transient ratio of wound surface and days for treatment were observed until epithelization was completed. As a control, a gauze soaked in sterile physiological saline was applied.

- 1) a rat experimental burn model: The back of a rat was dehaired, and a burn wound was evoked by pressing a heated iron under anesthesia. Dead skin was surgically removed 2 days after evoking the burn wound.
- 2) a burn wound of degree III: a model in which heat denaturation was reached to the subcutaneous tissue.

page 7: Bactericidal Activity (in vitro)

Growth of bacteria was not observed after 10, 30, 60, 90, 120 and 360 minutes in the case of MRSA, or after 10 and 360 minutes in the cases of bacterial strains other than MRSA, and bactericidal activity of Iodocoat<sup>®</sup> Ointment 0.9% was confirmed

bacterial strain	10 min	360 min
Bacillus subtilis	<10	<10
Escherichia coli	<10	<10
Staphylococcus aureus	<10	<10
Pseudomonas aeruginosa	<10	<10
Klebsiella pneumoniae	<10	<10
Proteus mirabilis	<10	<10
Acinetbacter baumannii	<10	<10
Candida albicans	<10	<10

bacterial strain	10 min	30 min
MRSA	<10	<10
	60 min	90 min
	<10	<10
	120 min	360 min
	<10	<10
		( 0 / 1)

(cfu/ml)

[Method] A sample solution was prepared by dissolving 10 g of Iodocoat<sup>®</sup> Ointment 0.9% in 240 ml of sterile physiological saline. A solution of inoculum strain was prepared by adjusting each concentration of bacteria solutions to 10<sup>7</sup> cfu/ml.

The solution of inoculum strain was added to a sample solution and viable bacteria count was measured by agar plate dilution method after 10, 30, 60, 90, 120 and 360 minutes in the

case of MRSA, or after 10 and 360 minutes in the cases of bacterial strains other than MRSA. In addition, period for contacting with bacteria was adjusted by neutralizing the activity of iodine by adding an aqueous solution of sodium thiosulfate.

### **Local Irritating Property**

Primary dermal irritation test

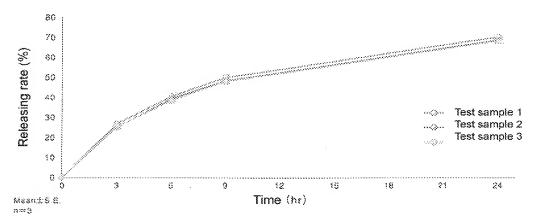
Primary dermal stimulating activity was evaluated by spreading the present ointment on a gauze and applied it (once a day, 24 hours, occlusive patch) on the healthy skin or damaged skin in the back of a rabbit. No skin reaction was observed in both of the healthy skin or damaged skin.

## Cumulative dermal irritation test

Cumulative dermal stimulating activity was evaluated by directly applying the present ointment (open application) or applying the same after being spread on a gauze (occlusive patch). No skin reaction was observed to the naked eye and a slight inflammatory cell infiltration was histopathologically detectable.

page 8: Iodine-releasing property

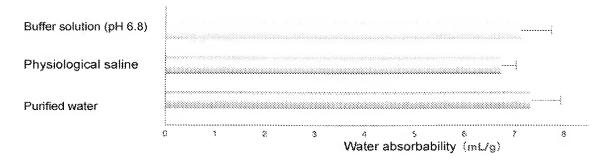
Iodocoat<sup>®</sup> Ointment 0.9% has a slow and sustained iodine-releasing property, and the averaged iodine-releasing rate was 68-70% after 24 hours.



[Method] Inner surface of a rotary basket (36 mesh) was covered with a filter paper and 0.5 g of Iodocoat<sup>®</sup> Ointment 0.9% was uniformly applied to it in a height of ca. 1 cm. It was rotated (25 rpm) in 100 mL of a releasing test solution containing potassium iodide at 30°C, and the amount of released iodine was measured by titration.

#### Water absorbability

Water absorbability of Iodocoat® Ointment 0.9% was not changed in a buffer solution(pH 6.8), physiological saline or purified water and sufficient water absorbability was confirmed.



[Method] 0.3 g of Iodocoat® Ointment 0.9% was spread on a gauze (4 cm x 4 cm) and the surface was covered with another gauze. 40 mL of a buffer solution(pH 6.8), physiological saline or purified water was added, left stand at room temperature for 24 hours and then water absorbability was calculated from the volume of unabsorbed solution.